PHARMACOKINETICS: ONE COMPARTMENT OPEN MODEL

I: I.V.BOLUS (Blood Level Data)

$$A = A_0 e^{-kt}$$

$$C = C_0 e^{-kt}$$

Logarithmic forms,

$$\log A = \log Ao - \frac{Kt}{2.303}$$

$$\log C = \log Co - \frac{Kt}{2.303}$$

II: I.V. BOLUS DOSE (URINARY EXCRETION DATA)

The first–order elimination rate constant can be computed from urine data by two methods

1. Rate of excretion method
2. Sigma –minus method

1. Rate of excretion method:

$$\therefore \frac{\Delta Au}{\Delta t} = k_e A_0 e^{-et}$$

Logarithmic form

$$\log \frac{\Delta Au}{\Delta t} = \log k_e A_0 - \frac{Kt}{2.303}$$

$$\log \frac{\Delta Au}{\Delta t} = \log keA_0 - \frac{k\,t_{midpo\,\mathrm{int}}}{2.303}$$

2. SIGMA MINUS METHOD:

$$A_u - Au = A_u^{\infty} e^{-kt}$$

Logarithmic form

$$\log\left(A_u^{\infty} - Au\right) = \log A_u^{\infty} - \frac{kt}{2.303}$$

$$\log ARE = \log A_u^{\infty} - \frac{kt}{2.303} \qquad \text{[ARE Plot]}$$

III. SHORT TERM CONSTANT RATE I.V, INFUSION-

$$C = \frac{ko}{VK}\,[\,1 - e^{-kt}\,]$$

IV. I.V. INFUSION-

$$C = \frac{ko}{VK}\,[\,1 - e^{-kt}\,]$$

$$\therefore C_{SS} = \frac{KO}{KV} \quad (\text{ since } e^{-kt} \approx 0, at\ t = \infty)$$

EXTRA VASCULAR ROUTE: other than i.v route (oral/rectal/im)

$$A = \frac{kaFD}{(ka-k)}[e^{-kt} - e^{-kat}]$$

Elimination Phase, $C = \frac{kaFD}{Vd\,(ka-k)}\,e^{-kt}$ [because A=Vd×C]

$$\text{LogC} = \frac{\log kaFD}{Vd(ka-k)} - \frac{Kt}{2.303}$$

Residual concentration, $Cr = \frac{kaFD}{Vd\,(ka-k)}\,e^{-kat}$

$$\text{LogCr} = \frac{\log kaFD}{Vd(ka-k)} - \frac{Kat}{2.303}$$

REPEATITIVE DOSING KINETICS – ONE COMPARTMENT

Intravenous(I.V.) REPETITIVE DOSING

$C = C_0.e^{-k\tau}$

$Cn = \frac{A0}{Vd}\,(\frac{1-e^{-nk\tau}}{1-e^{-k\tau}}) \times e^{-k\tau}$

Repetitive extravascular dosing-

$Cn = \frac{Ka\,.FD}{Vd(ka-kE)}\ (G.e^{-kt} - G.e^{-kat})$

$Cn = \frac{Ka\,.FD}{Vd(ka-kE)}\ [\frac{1-e^{-nk\tau}}{1-e^{-k\tau}}) \times e^{-k\tau} - \frac{1-e^{-nk\tau}}{1-e^{-k\tau}}) \times e^{-k\tau}\,]$ [since $G = (\frac{1-e^{-nk\tau}}{1-e^{-k\tau}})$]

$C\infty = \frac{A0}{Vd}\,(\frac{1}{1-e^{-k\tau}}).\,e^{-kt}$

$C\infty = \frac{A0}{Vd}\left(\frac{1}{1-e^{-k\tau}}\right).e^{-kt}$

$C\infty = \frac{Ka\ .FD}{Vd(ka-kE)}\left[\frac{1}{1-e^{-k\tau}}\ .\ e^{-kt}\ -\frac{1}{1-e^{-k\tau}}.\ e^{-kat}\right]$

Average $\underrightarrow{C\infty} = \frac{\boldsymbol{FD}}{\boldsymbol{Vd\ k\tau}}$

4.5 P : Biopharmaceutics & Pharmacokinetics- Practical

Study Material for Practical Class:

(for IV Pharm.D & I Pharm.D (PB)

Staff In- charge:

Dr P.K. Manna, Professor &

Dr. S. Madhusudhan, Associate Professor.

Determination of Renal Clearance

Number of methods are available:

1. From total elimination

$$Cl_r = \frac{FR + SR - RR}{C_p}$$

Where, FR = GFR= Glomerular Filtration Rate (mg/min)

SR = Active secretion rate (Tubular secretion) (mg/min)

RR = Reabsorption (Tubular Reabsorption) (mg/min)

Cp = Plasma concentration of drug (mg/ml)

2. From Elimination Rate constant

This is a very simple method, involves use of

K_E, V_d (in one compartment model)

β, V_d (in multi compartment model)

$Cl_r = K_E \cdot V_d$ (for one compartment model)

$Cl_r = \beta \cdot V_d$

Where, Ke = 0.693/ $t_{½}$ & Vd = Dose/ concn; β = 0.693/ $t_{½}$

Determination of Renal Clearance from Elimination Rate Constant

Ex No.1

Following 50 mg IV bolus dose of a drug, values of C_o and $t_{½}$ was determined as 1 mcg/ml and 6 hours respectively. Assuming the drug follows one compartment model; calculate renal clearance of the drug.

Solution:

$$Vd = \frac{Dose}{C_o} = \frac{50\ mg}{1\ mcg/ml} = \frac{50\ mg}{1\ mg/L} = 50\ L$$

$$K_E = \frac{0.693}{t_{½}} = \frac{0.693}{6} = 0.1155/\ hr$$

$Cl_r = K_E \cdot V_d$ = (0.1155/hr) (50 L) = 5.775 L/ hr

$$= \frac{5.775 \times 1000 \text{ ml}}{1 \times 60 \text{ min}} = 98.25 \text{ ml/min}$$

Determination of Renal Clearance from Urinary Excretion rate:

$$\text{Clearance} = \frac{\text{Urinary Excretion Rate}}{\text{Plasma Concentration}}$$

Urinary Excretion Rate = Clearance x Plasma Concentration

Measurement of Excretion Rate:
Administration of dose and collection of urine sample at predetermined time intervals
Determination of **volume and drug concentration** for each sample.

Calculation of **amount of drug excreted** during **each time interval**
(Volume of urine sample x drug concentration in sample)

Determine excretion rate

$$\text{Excretion Rate} = \frac{\text{Amount excreted during each time interval}}{\text{Time interval between urine collected}}$$

Measure of Plasma Concentration:

1. Select time intervals for determination of plasma drug concentration
2. (Selection is dictated by intervals selected for urine collection for measurement of Excretion rate)
3. Collect blood sample at selected time intervals (as Mid-point of corresponding excretion rate)
4. [To represent concentration of drug in plasma for a given excretion rate, it is essential to determine plasma drug concentration at the mid-point of corresponding excretion rate)
5. Determine drug plasma concentration

Determination of Clearance:

Plot excretion rate vs Plasma drug concentration at t mid-point of excretion rate.

Urinary Excretion Rate = Clearance x Plasma Concentration.

A graph is plotted with values of "Plasma Concentration (mg/L)" in X axis and "Excretion rate (mg/hr)" in Y axis.

The slope obtained from this graph indicates clearance of drug, since Clearance = Urinary Excretion rate / Plasma concentration.

$$\text{Clearance} = \frac{\text{Urinary Excretion Rate}}{\text{Plasma Concentration}} = \frac{\text{mg/hr}}{\text{mg/L}} = \frac{\text{L}}{\text{hr}} \quad (\text{or}) \quad \frac{\text{ml}}{\text{min}}$$

Ex. No.2: Determine Renal Clearance of Tetracycline from the Average Plasma Concentration and Urinary Excretion data, obtained after oral administration of a single 500mg dose to 8 human subjects, furnished in the Table below:

S. No	Time (Hours)	Plasma Concn (mg/L)	Amt. of drug excreted in urine (mg)
1	0	0	0
2	2	--	78
3	3	6.0	--
4	4	--	54
5	5	5.0	--
6	6	--	48
7	7	4.5	--
8	8	--	46
9	9	4.0	--
10	10	--	36
11	11	3.5	--
12	12	--	32
13	13	2.68	--
14	14	--	24
15	15	1.60	--
16	16	--	14
17	17	0.68	--
18	18	--	6

Re-tabulate data to obtain excretion rate and corresponding drug-plasma concentrations.

Interval	Amount excreted (mg)	Excretion Rate (mg/hr)	Mid-point (hr)	Drug plasma Concn(mg/L)
0 - 1	21 – 0 = 21	21/1 = 21	0.5	7.0
1 - 2	50 – 21= 29	29/1 = 29	1.5	9.6
2 - 4	94 – 50 =44	44/2 =22	3.0	7.4
4 - 8				
8 - 12				
12 - 20				
20 - 24	167-166 = 1	01/4 = 0.25	22	NA

Determination of Clearance:

Urinary Excretion Rate = Clearance x Plasma drug concentration.

Plot (Excretion Rate) vs (Plasma drug concentration) at $t_{mid\text{-}point}$ of excretion rate

Clearance = Slope of the line

This is only a sample calculation; students are requested to get points in slope in the graph (drawn from the slope of graph, plotted with values of "Plasma Concentration(mg/L)" in X axis and "Excretion rate (mg/hr)" in Y axis) to get more accurate results.

$$\text{Slope} = \frac{Y1 - Y2}{X1 - X2} = \frac{29 - 11}{9.6 - 3.8} = \frac{18}{5.8} = 3.103 \text{ L/ hr}$$

Since clearance is usually expressed as ml/min.

$$\text{Clearance (Clr)} = \frac{(3.103) \times (1000)}{60} \text{ ml/min} = 51.71 \text{ ml/min}$$

A linear relationship between excretion rate and concentration of drug in the plasma indicates clearance by Glomerular filtration because excretion rate was dependent on the plasma drug concentration. A value of 51.7 ml/min indicates the possibility of Reabsorption because normal renal clearance in healthy subjects ranges between 109 and 156 ml.imn.

Ex No. 3: Determine Renal Clearance of amoxicillin from the average Plasma concentration and Urinary excretion data, obtained after oral administration of a single 250 mg of amoxicillin to 6 human subjects, furnished in Table below:

S. No	Time (Hours)	Plasma Concn (mg/L)	Cumulative Amt. of drug excreted in urine (mg)
1	0	0	0
2	0.5	7.0	--
3	1.0	--	21
4	1.5	9.6	--
5	2.0	--	50
6	3.0	7.4	--
7	4.0	--	94
8	6.0	3.8	--
9	8.0	--	138
10	10	1.6	--
11	12	--	158
12	16	0.4	--
13	20	--	164
14	24	--	165

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REPEATITIVE DOSING KINETICS – ONE COMPARTMENT

Intravenous(I.V.) REPETITIVE DOSING

$C = C_0.e^{-k\tau}$

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Repetitive extravascular dosing-

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$C\infty = \frac{A0}{Vd}\left(\frac{1}{1-e^{-k\tau}}\right).e^{-kt}$

$C\infty = \frac{Ka\ .FD}{Vd(ka-kE)}\left[\frac{1}{1-e^{-k\tau}}\ .\ e^{-kt}\ -\frac{1}{1-e^{-k\tau}}.\ e^{-kat}\right]$

Average $\underrightarrow{C\infty} = \frac{\boldsymbol{FD}}{\boldsymbol{Vd\ k\tau}}$

STEADY STATE CONCENTRATIONS

I. RELATIONSHIP BETWEEN $C_{MAX\ (SS)}$ AND $C_{MIN\ (SS)}$

a. In case of IV administration:

The equations describing maximum and minimum drug-plasma concentrations at steady state following repetitive i.v. dosing:

$$C_{max\ ss} = \frac{Co}{1-e^{-K\tau}}; \qquad C_{min\ ss} = (C_{max\ ss})\, e^{-K\tau} = \frac{Co}{1-e^{-K\tau}} e^{-K\tau}$$

$$C_{max\ ss} - C_{min\ ss} = \frac{Co}{1-e^{-K\tau}} - \frac{Co}{1-e^{-K\tau}} e^{-Kt} = \frac{Co}{1-e^{-K\tau}} (1 - e^{-K\tau}) = C_0$$

In linear pharmacokinetics, since C_0 is a function of dose and apparent volume of distribution, the only factors that will influence C_0 are the dose and the apparent volume of distribution. So, as long as there is no change in the dose, the dosing interval and the apparent volume of distribution during multiple dosingthe difference between the maximum and minimum concentration of drug at steady-state will be always equal to C0 .

$C_{max\ ss} - C_{min\ ss} = C_0$, as long as Dose and V_d remains unchanged in multiple iv drug dosing.

b. In case of extravascular(EV) administration:

The equations describing maximum and minimum drug-plasma concentrations at steady state following repetitive extravascular dosing:

A simplified equation for $C_{max\ ss} = \frac{(B)\,(e^{-Ktmax})}{1-e^{-K\tau}}$

$$C_{min\ ss} = B\left(\frac{e^{-K\tau}}{1-e^{-K\tau}} - \frac{e^{-ka\tau}}{1-e^{-ka\tau}}\right)$$

in post absorptive phase, $C_{min\ ss} = B\left(\frac{e^{-K\tau}}{1-e^{-K\tau}}\right)$,

[since, in the post-absorptive phase the exponential term $e^{-ka\tau}$ approaches.]

t_{max} can be determined graphically if plasma profile exhibit a distinct and sharp peak. However, in absence of a sharp peak, t_{max} can be calculated using the following equation: $t_{max} = \frac{\ln ka - \ln Ke}{ka - Ke}$

In extravascular administration the difference between maximum and minimum concentrations of drug in plasma at steady-state, obtained by subtracting the above equations is a complex function of the factors that determine these concentrations.

In iv dosing : $C_{max\ ss} - C_{min\ ss} = C_0$, as long as Dose and V_d remains unchanged
In EV dosing: $C_{max\ ss} - C_{min\ ss}$ = a Complex Function of Factors determining Css

II. FACTORS AFFECTING STEADY STATE CONCENTRATIONS, $C_{MAX\ SS}$ AND $C_{MIN\ SS}$

A. EFFECT OF DOSE:

a. In case of IV administration:

For a dose, D, $C_{max\ ss} = \frac{Co}{1-e^{-K\tau}} = C_{max\ ss} = \frac{D}{V(1-e^{-K\tau})}$ [Since $C_0 = \frac{D}{V}$]

Similarly, for a dose D" = $C_{max\ ss"} = \frac{D"}{V(1-e^{-K\tau})}$

So, $\frac{\text{Cmax ss}}{\text{Cmax ss"}} = \frac{\frac{D}{V(1-e^{-K\tau})}}{\frac{D"}{V(1-e^{-K\tau})}} = \frac{D}{D"}$

Similarly, $\frac{\text{Cmin ss}}{\text{Cmin ss"}} = \frac{D}{D"}$

b. In case of Extravascular Administration:

Equations describing maximum and minimum plasma drug concentrations at steady state, $C_{max\ ss}$ and $C_{min\ ss}$, following extravascular drug administration are as follows:

$C_{max\ (ss)} = \frac{(B)\ (e^{-Ktmax})}{1-e^{-K\tau}}$; $C_{min\ (ss)} = B(\frac{e^{-K\tau}}{1-e^{-K\tau}} - \frac{e^{-ka\tau}}{1-e^{-ka\tau}})$ where, $B = \frac{FDka}{V(ka-K)}$

From the above Equations it is observed that during extravascular drug administration **$C_{min\ (ss)}$ depends on 3 factors**, B, K and **τ** while **$C_{max\ (ss)}$ depends on 4 factors**, B, K, **τ** and ka [Ref p361, Madan]

After extravascular administration of Dose, D, $C_{min\ (ss)} = \frac{FDka}{V(ka-K)}(\frac{e^{-K\tau}}{1-e^{-K\tau}} - \frac{e^{-ka\tau}}{1-e^{-ka\tau}})$

After extravascular administration of a new Dose, D", $C_{max\ (ss")} = \frac{FD"ka}{V(ka-K)}(\frac{e^{-K\tau}}{1-e^{-K\tau}} - \frac{e^{-ka\tau}}{1-e^{-ka\tau}})$

So, $\frac{\text{Cmin ss}}{\text{Cmin ss"}} = \frac{D}{D"}$ Similarly, $\frac{\text{Cmax ss}}{\text{Cmax ss"}} = \frac{D}{D"}$

B. EFFECT OF DOSING INTERVAL ON $C_{MAX\ (SS)}$ AND $C_{MIN\ (SS)}$:

a. In case of IV administration:

It is not as simple and straightforward as the effect of dose. In linear pharmacokinetics, the Dose Size affects only C_0 and C_0 α Dose Size (a linear relationship). As Dose Size and C_0 are directly proportional to each other, any change in dose size will have a linear effect on Css.

Dosing interval, τ, on the other hand is **not linearly related to** the terms used in calculation of minimum and maximum concentrations **($C_{min\ (ss)}$ and $C_{max\ (ss)}$)** at steady state (SS).

Dosing interval is related to 3 terms (the Persistance Factor, the Loss Factor and the Accumulation Factor) and these terms are **not linearly related to Dosing Interval**. Additionally, Dosing Interval aopears in the exponential terms of these of these three factors. As a result, the relationship between Dosing Interval and Css is much complicated and not possible to relate them with a simple relationship.

b. In case of Extravascular administration:

In case of extravascular dosing also, for similar reasons, the effect of Dosing Interval on Cmin(ss) and Cmax(ss) is not simple and straightforward. Hence, it is not possible to relate the effect of dosing interval on Css with a simple relationship.

Effect of Dose on Css for both ivand Extravascular drug administration

$$\frac{D}{D''} = \frac{\text{Cmin ss}}{\text{Cmin ss}''} = \frac{\text{Cmax ss}}{\text{Cmax ss}''}$$

Relationship between Dosing Interval and Css is much complicated and not possible to relate them with a simple relationship.

Excercise: Following single i.v. bolus dosing of 50 mg of a drug, C_0 and K were determined as follows: C_0 = 2.0mcg/ml and K = 0.115/hr.

Find out Css(min) and Css(max) when a) D = 50 mg, τ = 8.0 hrs

and b) D = 50 mg, τ = 8.0 hrs

Solution:

Exercise: Plasma con. vs time data obtained following a single oral dose are furnished below:

Sl.No.	1	2	3	4	5	6	7	8	9
Time (hr)	0.5	1.0	1.5	2.0	3.0	4.0	5.0	6.0	7.0
PlasmaConcn (mcg/ml)	19.8	27.0	28.3	28.0	23.7	20.0	16.7	14.0	12.0

Calculate Cmax(ss) and Cmin(ss) of the drug, if 500 mg of drug is administered repeatedly every 4 hrs.

Solution:

IV PHARMD & I PHARMD POST BACCALAUREATE

COURSE_ 4.5: BIOPHARMACEUTICS AND PHARMACOKINETICS

UNIT: 7: BIOAVAILABILITY AND BIOEQUIVALENCE

BASIC CONCEPT, FACTORS AND TERMINOLOGY:

BIOAVAILABILITY: Bioavailability is an absolute term that indicates measurement of both the true rate ant total amount (extent) of drug that reaches the systemic (general) circulation from an administered dosage form.

Absolute Bioavailability: When the systemic availability of a drug administered orally is determined in comparison to its intravenous administration, it is called as absolute bioavailability and denoted by F.

$$\text{Absolute bioavailability, } F = (AUC)_{Oral//}/(AUC)_{i.v.}$$

Relative Bioavailability: When the systemic availability of a drug after oral administration is compared with that of an oral standard of the same drug (such as an aqueous or non-aqueous solution or a suspension), it is refereed to as relative or comparative bioavailability).

$$\text{Absolute bioavailability, } F = (AUC)Text_{Oral//}/(AUC)_{Reference}$$

EQUIVALENCE: is a relative term that compares one drug product with another or with a set of established standards. Equivalence may be defined in se3veral ways –

i. Chemical\equivalence indicates that two or more dosage forms contain the same labeled quantities of the drug.

ii. Pharmaceutical equivalece means two or more brands of the same dosage form contain same strength or concentration of the same active ingredient(s) (e.g., Paracetamol 400 mg tablets).

iii. Clinical equivalence means the same drug from two or more dosage forms gives identical *in vivo* effects (pharmacological response or control of a symptom or disease.

iv. Therapeutic equivalence means two structurally different chemicals give same clinical results.

v. Bioequivalence means a drug in two or more similar dosage forms reaches the systemic circulation at the same relative rate and extent, ie. The plasma level profiles of the drugs are superimposable within acceptable limits of variation**.**

Bioequivalence is defined as the application of the concept of bioavailability in determining whether the systemic availability of a test and a recognized standard drug product containing equal doses of the same drug are equivalent or not.

Bioequivalecy study is an extension of the concept of relative bioavailability study .It involves comparing the bioavailabilty of a particular drug from a test product and a recognized standard dosage form to find out whether both the products containing equal doses of the same drug are equivalent or not in terms of their systemic availability (rates and extents of absorption).

Bioequivalence studies are used to assess the expected *in-vivo* biological equivalence of two proprietary preparations of a drug.

If a new product is intended to be a substitute for an approved medicinal product as a pharmaceutical equivalent/ alternative, the bioequivalence studies should be carried out.

PARAMETERS FOR DESCRIBING BIOEQUIVALENCY (Determinant Pharmacokineti c Parameters of Bioequivalency): Cmax, Tmax, AUC

Equivalence Criteria:

THE ±20 RULE: Bioequivalence is concluded if the average bioavailability of the test formulation is within ±20% of that of the reference formulation

THE 80/125 RULE: Bioequivalence is concluded if the average bioavailability of the test formulation is within (80%, 125%) that of the reference formulation.

FACTORS AFFECTING BIOAVAILABILITY

Three major factors -

- Pharmaceutical factors
- Patient related factors
- Route of administration

NEED FOR BIOAVAILABILITY STUDIES:

- To evaluate the absolute systemic availability of active drug substance from a dosage form.
- To determine the linearity of the bioavailability parameters over the proposed clinical dosage range.
- To estimate the inter and intra subject variability.
- To study the effect of food on bioavailability

GOALS OF BIOAVAILABILITY STUDIES

- Determination of influence of excipients, patient-related factors and possible interaction with other drugs on the efficacy of absorption.
- Primary stages of development of a suitable dosage form for a new drug entity to obtain evidence of therapeutic utility.
- Comparison of availability of a drug substance from different dosage forms or from the same dosage form produced by different manufacturers.
- Control of quality of a drug product during the early stages of marketing in order to determine the influence of processing factors, storage and stability on drug absorption

BIOAVAILABILITY STUDIES – TWO TYPES:

First Type—For Assessment of the bioavailability of a new drug formulation

Steps:

Pharmacokinetic parameters following different routes of administration of the new drug are obtained. → Utilization in developing optimum dosage regimen → Formulation of a new drug suitably for an intended route of administration. → Assessment of bioavailability.

Second Type--- For Comparison of a test formulation with that with that of a reference standard dosage form which is proved to have therapeutic efficacy and safety. Also known as **bioequivalence studies**.

METHODS:

PHARMACOKINETIC METHODS	PLASMA LEVEL TIME STUDIES URINARY EXCRETION STUDIES
PHARMACODYANAMIC	ACUTE PHARMACOLOGICAL

METHODS	RESPONSE THERAPEUTIC RESPONSE

Plasma Level- Time Studies:

Plasma Level- Time Studies A profile is constructed showing the concentration of drug in blood at the specific times the samples were taken. Bioavailability (the rate and extent of drug absorption) is generally assessed by the determination of following three parameters.

They are

1. C max (Peak plasma concentration)
2. t max (time of peak)
3. Area under curve

Plasma Drug Concentration- Time Profile Plasma Drug Concentration-Time Profile :

Plasma Drug Concentration- Time Profile Plasma Drug Concentration-Time Profile A topical plasma concentration- time profile showing pharmacokinetic and pharmacodynamic parameters obtained after oral administration of single dose of a drug.

Peak plasma concentration & Time of peak plasma concentration:

Peak plasma concentration & Time of peak plasma concentration C max (Peak plasma concentration): Maximum concentration of the drug obtained after the administration of single dose of the drug. Expressed in terms of µg /ml or mg/ml t max (Time of peak plasma concentration): Time required to achieve peak concentration of the drug after administration. Gives indication of the rate of absorption. Expressed in terms of hours or minutes.

Area under curve:

Area under curve the plasma level-time curve that gives a measure of the extent of absorption or the amount of drug that reaches the systemic circulation. The extent of bioavailability can be determined by following equations: For signal dose study: For multiple dose study.

BIOAVAILABILITY STUDY PROTOCOL:

Introduction : Bioavailability studies are conducted to evaluate the performance of the dosage forms. It is usually conducted in normal healthy adults under standardized conditions. Normally, single doses of the test and reference/standard drug products are evaluated. In selected cases, multiple-dose regimens must be evaluated, eg. Acid-labile drugs.

Bioavailability study is conducted in normal healthy adults volunteers under standardized conditions.

NUMBER OF DOSES:

Usually, single doses of each of the test and the standard (Reference) product is used in the study. In selected cases, multiple-dose regimens are used, eg. Acid labile drugs.

STUDY GROUP: 12 to 24 healthy volunteers

Volunteer selection criteria- to minimize individual variation –

Inclusion Criteria:

i). Age: 18 – 50 yrs

ii). Body weight: 54 to 91 kg [within 10% of ideal body weight (IBW)]

[IBW (men) = 50 kg ± 1 kg per every 2.5 cm height above or below 150 cm.

IBW (women) = 45 kg ± 1 kg per every 2.5 cm height above or below 150 cm.

Percent Fat = 90 – 2(Height – Girth) inches

[Girth = measurement around the waist, using umbilical level at exhalation]

iii. Medical History: within acceptable medical history – no recent history of

iv. Medication history: Individuals without recent medication history.

Exclusion Criteria:

i. Medical History: volunteers with history of diseases like –

iv. Medication history: Individuals with history recent/present medication that may alter blood level of the drug under test.

STUDY CONDITIONS:

i). drug free period prior to testing/dose administration: not less than TWO WEEKS to eliminate drug effect on liver enzymes.

ii). Period of Fasting prior to dosing: usually overnight with free access to water.

iii). Period of Fasting after dosing: usually 2 to 4 hrs.; only standard meal should be given 2 to 4 hrs after dosing.

STUDY DESIGN: Complete Crossover Design is used to minimize variation between the test and the standard products..

DOSING AND SAMPLING SEQUENCE:

Sequence Effect – Sequential Sequence should not be followed to avoid bias due to sequence effect.

Randomly designed sequence following a LATIN SQUARE DESIGN should be followed to remove bias.

STUDY METHODOLOGY:

In a study with two products –

one is STANDARD PRODUCT and the other TEST PRODUCT. Each volunteer (subject) receives each of the products (standard and test) in TWO Stages, stage I and stage II.

Grouping of the volunteers: selected subjects are divided into two equal groups homogeneously (as closely as possible): Gp I and Gp II.

STAGE I: Initial period of Dosing and Sampling –

Dosing: Gp I receives STANDARD PRODUCT and Gp **II, receives** TEST PRODUCT

WASHOUT P0ERIOD: Normally 10 half-lives (t½):

At least 99.9% of administered dose should be eliminated before administration of the 2nd product, to minimize CARRY OVER EFFECT of the drug or its metabolite into the next stage, Stage II, of the study.

STAGE II: Post washout period of Dosing and Sampling –

Dosing: Gps are crossed over ie. The gp that received standard product in Stage I, receives test product in stage II and *vice versa* with the other gp.

Gp II receives STANDARD PRODUCT

Gp I receives TEST PRODUCT

SINGLE DOSE CROSS OVER STRUDY DESIGN

Group Number of Volunteer	**STAGE I (Initial period)**	**WASH OUT PERIOD Normally 10 t½**	**STAGE II (Post washout period)**
GROUP I	**STANDARD PRODUCT**		**TEST PRODUCT**
GROUP II	**TEST PRODUCT**		**STANDARD PRODUCT**

Frequency and Duration of Sampling:

Interval of sampling: should be identical in both the stages

Frequency: Adequate to define – Absorption Phase, Peak Concn. and Elimination Phase

At least 2 to 3 experimental points (excluding zero time point) in each of the Absorption and Elimination Phases.

Duration: 3 to 5 Half-lives (t½) ie. Until 87.5% to 96.9% of administered dose is eliminated or until plasma levels fall to 5 to 10 % of the peak concn.

ASSAY PROCEDURE: Sample treatment, Sample storage condition and Assay method Should be identical in both the stages.

Bioanalytical method should be - i. Sensitive enough to analyse the drug in biosample at very low concentrationand and ii) reliable, accurate & precise.

When the samples can not be analyzed immediately, they should be stored frozen at – 20 ºC to – 70 ºC, depending on stability. Before storage, the samples should be centrifuged to separate plasma or serum to minimize "Blood storage effect". However, the samples should be analyzed before any degradation. Information on stability of drugs & the metabolites in bio-samples is necessary in planning for analysis.